

Exhibit 18



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(54) **FRUCTOSYLTRANSFERASES**

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(30) **Foreign Application Priority Data**

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(58) Field of Search 435/97, 101, 193, 435/252.9

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G.H. van Geel-Schutten et al. "Biochemical and Structural Characterization of the Glucan and Fructan Exopolysaccharides Synthesized by the *Lactobacillus reuteri* Wild-Type Strain and by Mutant Strains", Applied Environ. Microbiol. 65(7); 3008-3014. (Jul. 1).*

G.H. van Geel-Schutten et al. "Screening and Characterization of Lactobacillus Strains Producing Large Amounts of Exopolysaccharides", Appl. Microbiol. Biotechnol. 50: 697-703. (1998).*

* cited by examiner

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(57) **ABSTRACT**

The present invention describes two novel proteins having fructosyltransferase activity. Both enzymes are derived from lactobacilli, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins produces an inulin and fructo-oligosaccharides, while the other produces a levan and fructo-oligosaccharides. According to the invention lactobacilli capable of producing an inulin and/or a levan and/or fructo-oligosaccharides using one or both of the fructosyltransferases can be used as a probiotic or a symbiotic.

8 Claims, 8 Drawing Sheets

Fig 1 (1)

1 ~~tacaatgggg tgaaggaggt gaagaaacgg~~ ggttacttct atgctagaac gcaaggaaca 19ftf>

y n g v a e v k k r g y f y a r t
y n g v a e v n t e r q a n g q i

61 taaaaaaatg tataaaagcg gtaaaaattg ggcagtcggt acactctega ctgctgcgct

1 m y k s g k n v a v v t l s t a a

121 ggtatttgggt gcaacaactg taatgcctc cgcggacaca aatattgaaa acaatgattc

18 l v f g a t t v n a s a d t n i e n n d

181 ttctactgta caagttacaa caggtgataa tgatattgct gttaaaagtg tgacacttgg

38 s s t v q v t t g d n d i a v k s v t l

241 tagtggctaa gttagtgcag ctagtgtac gactattaga acttctgcta atgcaaatag

58 g s g q v s a a s d t t i r t s a n a n

301 tgcttcttct gccgctaata cacaaaattc taacagtcac gtatgaagtt ctgctgcaat

78 s a s s a a n t q n s n s q v a s s a a

361 aacatcatct acaagttcgg cagcttcatt aaataacaca gatagtaaag cggctcaaga

98 i t s s t s s a a s l n n t d s k a a q

421 aaatactaata acagccaaaa atgatgacac gcaaaaagct gcaccagcta acgaatcttc

118 e n t n t a k n d d t q k a a p a n e s

481 tgaagctaaa aatgaaccag ctgtaaacgt taatgattct tcagctgcaa aaaatgatga

138 s e a k n e p a v n v n d s s a a k n d

541 tcaacaatcc agtaaaaaga atactaccgc taagttaaac aaggatgctg aaaacgttgt

158 d q q s s k k n t t a k l n k d a e n v

601 aaaaaaggcg ggaattgac etaacagttt aactgatgac cagattaaag cattaaataa

178 v k k a g i d p n s l t d d q i k a l n

Fig 1 (2)

661 gatgaacttc tggaaagctg caaagctctgg tacacaaatg acttataatg atttccaaaa
198 k m n f s k a a k s g t q m t y n d f q

721 gattgctgat ~~acgttatctca~~ ~~aaagagatgg~~ ~~tgggtacaca~~ gttccattct ttaaagcaag 20ftfi <
210 k i a d t l i k q d g r y t v p f f k a

781 tgaatcaaaa aatatgcctg ccgtacaaac taaagatgca caaactaata ctattgaacc
238 s e i k n m p a a t t k d a q t n t i e

841 tttagatgta tgggattcat ggccagttca agatgttcgg acaggacaaag ttgctaattg 5ftf >
258 p l d v w d s v p v q d v r t g q v a n 8ftfi <

901 gaatggctat caacttgta tgcgaatgat ggggaattcca aaccanaatg ataactat
270 w n g y q l v i a m m g i p n q n d n b

961 ctatctctta tataataagt atggtgataa tgaattaagt cattggaaga ~~atgtaggctc~~ 7ftf >
298 i y l l y n k y g d n e l s h v k n v g

1021 ~~aatttttggc~~ tataattcta ccgcggttcc ~~acagagatgg~~ ~~tccggatcag~~ ~~ctgttttgaa~~ 7ftf >
318 p i f g y n s t a v s q e v s g s a v l 6ftfi <

1081 cagtgaatac tctatccaat tatttttatc aagggtagac acgtctgata acaataccaa
338 n s d n s i q l f y t r v d t s d n n t

1141 tcatcaaaaa attgctagcy ctactcttta ttttaactgat ~~antatggaa~~ ~~atgtatcaact~~ NheI
350 n h q k i a s a t l y l t d n n g n v s AC1(i)<>

1201 cgtccagga cgaatgact atattgtatt tgaagggtgat ~~ggtatattact~~ ~~accanaactta~~ AC2(i)<>
378 l a q v r n d y i v f e g d g y y y q t

1261 ~~tgtcaantgg~~ aaagctacta acaaaggtgc cgataatatt gcaatgcgtg atgctcatgt
398 y d q w k a t n k g a d n i a m r d a h

Fig 1 (3)

1321 aattgaagat ggtaatgggt atcggtacct tgtttttgaa gcaagtactg gtttggaaaa
418 v i e d g n g d r y l v f e a s t g l e

1381 ttatcaagcg gaggaccaaa ttataactg gttaaattat ggcygagatg acgcatttaa
438 n y q g e d q i y n v l n y g g d d a f

1441 tatcaagagc ttatttagaa ttctttccaa tgatgatatt aagagtcggg caacttgggc
458 n i k s l f r i l s n d d i k s r a t v

1501 taatgcagct atcggtatcc tcaaaactaaa taaggacgan aagaactcta aggtggcaga
478 a n a a i g i l k l n k d e k n p k v a

1561 gttatactca ccaktaattt ctgcaccaat ggttaagcat gaaattgagc gaccaaatgt
498 e l y s p l i s a p n v s d e i e r p n

1621 agttaaatta ggttaataaat attacttatt tgcgcctacc cytttaaatc gaggaagtaa
518 v v k l g n k y y l f a a t r l n r g s

1681 tgatgatgct tggatgaatg ctaattatgc cgttggtgat aatgttgcaa tggtcggata
538 n d d a v m n a n y a v g d n v a n v g

1741 tgttgctgat agtctaactg gatcttataa gccattaaat gattctggag tagtcttgac
558 y v a d s l t g s y k p l n d s g v v l

1801 tgcttctgtt cctgcnaact ggcygacagc aacttattca tattatgctg tcccggtgc
578 t a s v p a n v r t a t y s y y a v p v

1861 cggaaaagat gaccaagtat tagttacttc atatatgact aatagaaatg gagtgcggg
598 a g k d d q v l v t s y n t n r n g v a

1921 taaaggaatg gattenactt gggcaacgag ttcttacta caaattaacc cggataaacac 12frfi <
618 g k g m d s t v a p s f l l q i n p d n

Fig 1 (4)

1981 aactactgtt ttactataaa tgactaatca aggggattgg atttgggatg attcaagcga
638 t t t v l a k m t n q g d w i w d d s s

2041 aaatcttgat atgattggtg atttagactc cgtctcttta cctggcgaac gtgataaacc
658 e n l d m i g d l d s a a l p g e r d k

2101 tgttgattgg gacttaattg gttatggatt aaaaccgcat gatcctgcta caccaaatga
678 p v d w d l i g y g l k p h d p a t p n

2161 tcctgaaacg ccaactacac cagaaccccc tgagacacct aatactccca aaacacccaa
698 d p e t p t t p e t p e t p n t p k t p

2221 gactcctgaa aatcctggga cacctcaaac tcctaataca cctaatactc cygaaattcc
718 k t p e n p g t p q t p n t p n t p e i

2281 tttaactcca gaaacgccta agcaacctga aacccaacct aataatcgtt tgccacaaac
738 p l t p e t p k q p e t q t n n r l p q

2341 tggaaataat gccataaag ccattgattgg cctaggtatg ggaacattgc ttagtatggt
758 t g n n a n k a m i g l g m g t l l s m

2401 tggctcttgc gaaattaaca aacgtcgatt taactaata cttaaataa aaacgcgtaa
778 f g l a e i n k r r f n

2461 gccttaaatc cagcttaacg gttttttatt ttaaaagttt ttattgtaaa aaagogaatt

2521 atcataata ctatgcaat tgttgtaaga ccttacgaca gtagtaacaa tgaatttgcc

2581 catctttgtc gg

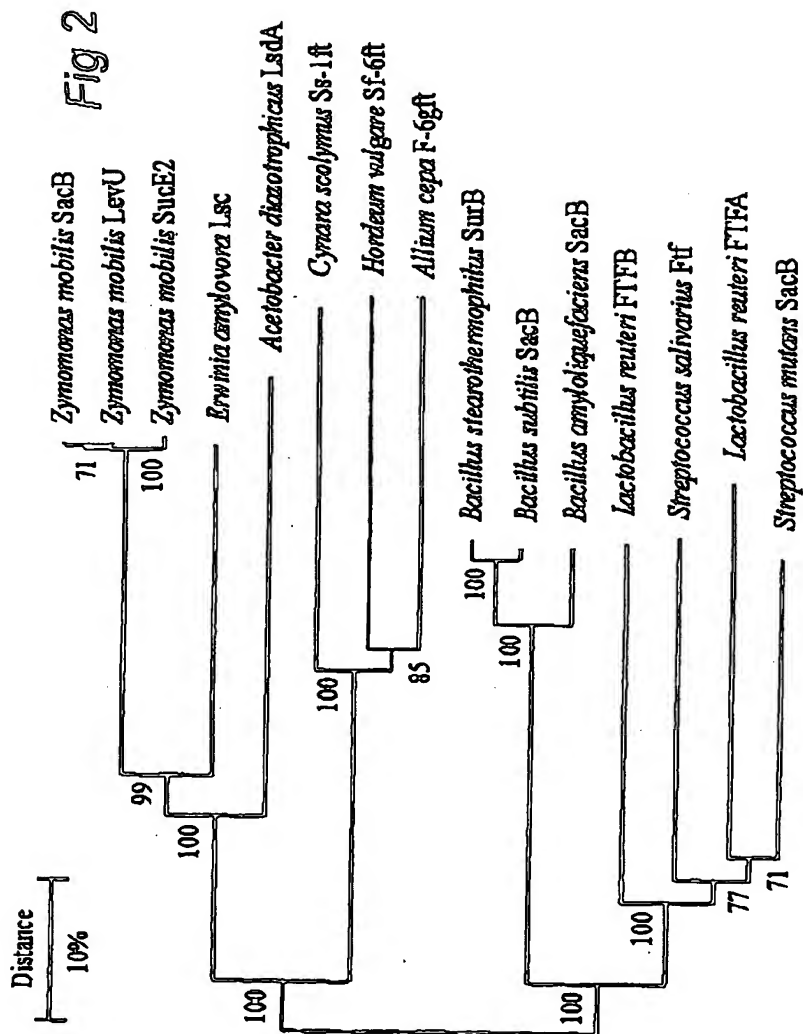


Fig 3

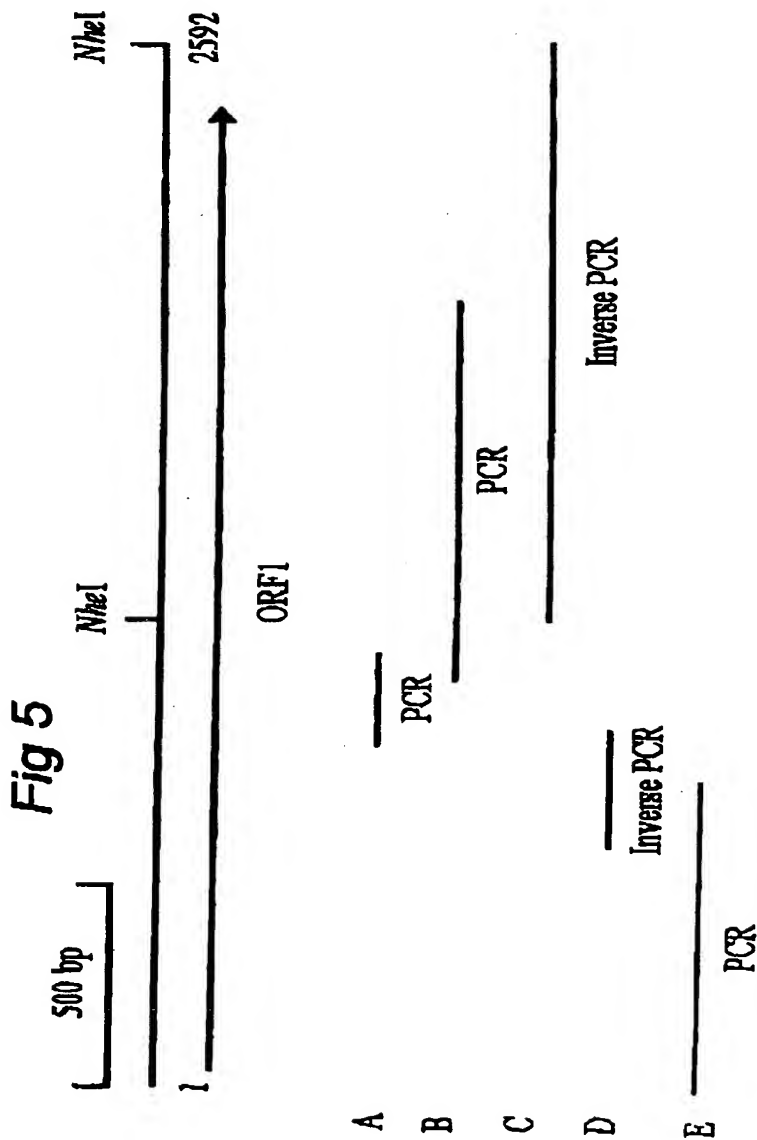
The N-terminal sequence of FTFB (levansucrase):

(A) Q V E S N N Y N G V A E V N T E R Q A N G Q I (G) (V) (D).

Internal peptide sequences of FTFB (levansucrase):

- (M) (A) H L D V W D S W P V Q D P (V),
- N A G S I F G T (K),
- V (E) (E) V Y S P K V S T L M A S D E V E.

5ttf				
<i>B. amyloliquefaciens</i> SacB	80	GLDVWDSWFLQNAD	93	
<i>B. subtilis</i> SacB	82	GLDVWDSWFLQNAD	95	
<i>S. mutans</i> SacB	243	DLDVWDSWFLQDAK	256	
<i>S. salivarius</i> Ftf	282	KIDVWDSWFLQDAK	295	
		:*****:~:~.		
6ttf1				
<i>B. amyloliquefaciens</i> SacB	156	QTQWNSGBATFTSDGK	171	
<i>B. subtilis</i> SacB	158	QTQWNSGBATFTSDGK	173	
<i>S. mutans</i> SacB	312	LTQWNSGBATVNIEDGS	327	
<i>S. salivarius</i> Ftf	351	DDQQWNSGBATVNSDGS	366	
		*:*****...*~.		
12ttf1				
<i>B. amyloliquefaciens</i> SacB	440	KATFGPSFLMN	450	
<i>B. subtilis</i> SacB	440	QSTFAPSFLIN	450	
<i>S. mutans</i> SacB	609	NSTWAPSFLIQ	619	
<i>S. salivarius</i> Ftf	655	KSTWAPSFLIK	665	
		:~:~.*****:		



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FRUCTOSYLTRANSFERASES

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of application of U.S. application Ser. No. 09/604,958 filed on Jun. 28, 2000, now U.S. Pat. No. 6,635,460, which claims priority from European Application No. 00201872.9 filed on May 25, 2000.

The present invention is in the field of enzymatic production of biomolecules. The invention is particularly concerned with two novel fructosyltransferases derived from lactobacilli and with a process for recombinant production of the enzymes and for the production of useful levans, inulins and fructo-oligosaccharides from sucrose.

BACKGROUND OF THE INVENTION

Lactic acid bacteria (LAB) play an important role in the fermentative production of food and feed. Traditionally, these bacteria have been used for the production of for instance wine, beer, bread, cheese and yoghurt, and for the preservation of food and feed, e.g. olives, pickles, sausages, sauerkraut and silage. Because of these traditional applications, lactic acid bacteria are food-grade microorganisms that possess the Generally Recognised As Safe (GRAS) status. Due to the different products which are formed during fermentation with lactic acid bacteria, these bacteria contribute positively to the taste, smell and preservation of the final product. The group of lactic acid bacteria encloses several genera such as *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, etc.

In recent years also the health promoting properties of lactic acid bacteria have received much attention. They produce an abundant variety of exopolysaccharides (EPS's). These polysaccharides are thought to contribute to human health by acting as prebiotic substrates, nutraceuticals, cholesterol lowering agents or immunomodulants.

To date high molecular weight polysaccharides produced by plants (such as cellulose, starch and pectin), seaweeds (such as alginate and carrageenan) and bacteria (such as alginate, gellan and xanthan) are used in several industrial applications as viscosifying, stabilising, emulsifying, gelling or water binding agents. Although all these polysaccharides are used as food additives, they originate from organisms not having the GRAS status. Thus they are less desirable than the exopolysaccharides of microorganisms, such as lactic acid bacteria, which have the GRAS status.

The exopolysaccharides produced by LAB can be divided in two groups, heteropolysaccharides and homopolysaccharides; these are synthesized by totally different mechanisms. The former consist of repeating units in which residues of different types of sugars are present and the latter consist of one type of monosaccharide. The synthesis of heteropolysaccharides by lactic acid bacteria, including lactobacilli, has been studied extensively in recent years. Considerably less information is available on the synthesis of homopolysaccharides from lactobacilli, although some studies have been performed. Homopolysaccharides with fructose as the constituent sugar can be divided into two groups, inulins and levans. Inulins consist of 2,1-linked β -fructofuranoside residues, whereas levans consist of 2,6-linked β -fructofuranoside residues. Both can be linear or branched. The size of bacterial levans can vary from 20 kDa up to several MDa. There is limited information on the synthesis of levans. In most detail this synthesis has been studied in *Zymomonas mobilis* and in *Bacillus* species.

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Within lactic acid bacteria, fructosyltransferases have only been studied in streptococci. So far no fructosyltransferases have been reported in lactobacilli.

In a recent report the *Lactobacillus reuteri* strain LB 121 was found to produce both a glucan and a fructan when grown on sucrose, but only a fructan when grown on raffinose (van Geel-Schutten, G. H. et al., Appl. Microbiol. Biotechnol. (1998) 50, 697-703). In another report the glucan and fructan were characterised by their molecular weights (of 3,500 and 150 kDa respectively) and the glucan was reported to be highly branched with a unique structure consisting of a terminal, 4-substituted, 6-substituted, and 4,6-disubstituted α -glucose in a molar ratio 1.1: 2.7:1.5:1.0 (van Geel-Schutten, G. H. et al., Appl. Environ. Microbiol. (1999) 65, 3008-3014). The fructan was identified as a linear (2 \rightarrow 6)- β -D-fructofuranan (also called a levan). This was the first example of fructan synthesis by a *Lactobacillus* species.

SUMMARY OF THE INVENTION

Two novel genes encoding enzymes having fructosyltransferase activity have now been found in *Lactobacillus reuteri*, and their amino acid sequences have been determined. These are the first two enzymes identified in a *Lactobacillus* species capable of producing a fructan. One of the enzymes is an inulosucrase which produces a high molecular weight (>10⁷ Da) fructan containing β (2-1) linked fructosyl units and fructo-oligosaccharides, while the other is a levansucrase which produces a fructan containing β (2-6) linked fructosyl units. The invention thus pertains to the enzymes, to DNA encoding them, to recombinant cells containing such DNA and to their use in producing carbohydrates, as defined in the appending claims.

DESCRIPTION OF THE INVENTION

It was found according to the invention that one of the novel fructosyltransferases (FTFA; an inulosucrase) produces a high molecular weight inulin with β (2-1) linked fructosyl units and fructo-oligosaccharides. The fructo-oligosaccharides synthesis was also observed in certain *Lactobacillus* strains, in particular in certain strains of *Lactobacillus reuteri*. However, the inulin has not been found in *Lactobacillus reuteri* culture supernatants, but only in extracts of *E. coli* cells expressing the above-mentioned fructosyltransferase. This inulosucrase consists of either 798 amino acids (2394 nucleotides) or 789 amino acids (2367 nucleotides) depending on the potential start codon used. The molecular weight (MW) deduced of the amino acid sequence of the latter form is 86 kDa and its isoelectric point is 4.51, at pH 7.

The amino acid sequence of the inulosucrase is shown in SEQ ID No. 1 (FIG. 1, amino acid residues 1-789). As mentioned above, the nucleotide sequence contains two putative start codons leading to either a 2394 (see SEQ ID No. 3) or 2367 (see SEQ ID No. 2) nucleotide form of the inulosucrase. Both putative start codons are preceded by a putative ribosome binding site, GGGG (located 12 base pairs upstream its start codon) or AGGA (located 14 base pairs upstream its start codon), respectively (see FIG. 1 and SEQ ID No. 4).

The present invention covers a protein having inulosucrase activity with an amino acid identity of at least 65%, preferably at least 75%, and more preferably at least 85%, compared to the amino acid sequence of SEQ ID No. 1. The invention also covers a part of a protein with at least 15 contiguous amino acids which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 1.

Fructosyltransferases have been found in several bacteria such as *Zymomonas mobilis*, *Erwinia amylovora*, *Acetobacter amylovora*, *Bacillus polymyxa*, *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus*, and *Bacillus subtilis*. In lactic acid bacteria this type of enzyme previously has only been found in some streptococci. Most bacterial fructosyltransferases have a molecular mass of 50–100 kDa (with the exception of the fructosyltransferase found in *Streptococcus salivarius* which has a molecular mass of 140 kDa). Amino acid sequence alignment revealed that the novel inulosucrase of lactobacilli has high homology with fructosyltransferases originating from Gram positive bacteria, in particular with *Streptococcus* enzymes. The highest homology (FIG. 2) was found with the SacB enzyme of *Streptococcus mutans* Ingbritt A (62% identity within 539 amino acids).

Certain putative functions based on the alignment and site-directed mutagenesis studies can be ascribed to several amino acids of the novel inulosucrase. Asp-263, Glu-330, Asp-415, Glu-431, Asp-511, Glu-514, Arg-532 and/or Asp-551 of the amino acid sequence of SEQ ID No. 1 are identified as putative catalytic residues. Noteworthy, a hydrophobicity plot according to Kyte and Doolittle (1982) J. Mol. Biol. 157, 105–132 suggests that the novel inulosucrase contains a putative signal sequence according to the Von Heijne rule. The putative signal peptidase site is located between Gly at position 21 and Ala at position 22. Furthermore, it is striking that the C-terminal amino acid sequence of the novel inulosucrase contains a putative cell wall anchor amino acid signal LPXTG (SEQ ID No. 5) and a 20-fold repeat of the motif PXX (residues 690–749 of SEQ ID NO: 1) (see figure 1), where P is proline and X is any other amino acid. In 15 out of 20 repeats, however, the motif is PXT. This motif has so far not been reported in proteins of prokaryotic and eukaryotic origin.

A nucleotide sequence encoding any of the above mentioned proteins, mutants, variants or parts thereof is also a subject of the invention. Furthermore, the nucleic acid sequences corresponding to expression-regulating regions (promoters, enhancers, terminators) of at least 30 contiguous nucleic acids contained in the nucleic acid sequence (-67)-(-1) or 2367–2525 of SEQ ID No. 4 (see also FIG. 1) can be used for homologous or heterologous expression of genes. Such expression-regulating sequences are operationally linked to a polypeptide-encoding nucleic acid sequence such as the genes of the fructosyltransferase according to the invention. A nucleic acid construct comprising the nucleotide sequence operationally linked to an expression-regulating nucleic acid sequence is also covered by the invention.

A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The inulosucrase gene (starting at nucleotide 41) has been cloned in an *E. coli* expression vector under the control of an ara promoter in *E. coli* Top10. *E. coli* Top10 cells expressing the recombinant inulosucrase hydrolysed sucrose and synthesized fructan material. SDS-PAGE of arabinose induced *E. coli* Top10 cell extracts suggested that the recombinant inulosucrase has a molecular weight of 80–100 kDa, which is in the range of other known fructosyltransferases and in line with the molecular weight of 86 kDa deduced of the amino acid sequence depicted in FIG. 1.

The invention further covers an inulosucrase according to the invention which, in the presence of sucrose, produces a inulin having $\beta(2-1)$ -linked D-fructosyl units and fructo-

oligosaccharides. Two different types of fructans, inulins and levans, exist in nature. Surprisingly, the novel inulosucrase expressed in *E. coli* Top10 cell synthesizes a high molecular weight ($>10^7$ Da) inulin and fructo-oligosaccharides, while in *Lactobacillus reuteri* culture supernatants, in addition to the fructo-oligosaccharides, a levan and not an inulin is found. This discrepancy can have several explanations: the inulosucrase gene may be silent in *Lactobacillus reuteri*, or may not be expressed in *Lactobacillus reuteri* under the conditions tested, or the inulosucrase may only synthesize fructo-oligosaccharides in its natural host, or the inulin polymer may be degraded shortly after synthesis, or may not be secreted and remains cell-associated, or the inulosucrase may have different activities in *Lactobacillus reuteri* and *E. coli* Top10 cells.

It was furthermore found according to the invention that certain lactobacilli, in particular *Lactobacillus reuteri*, possess another fructosyltransferase, a levansucrase (ITFB), in addition to the inulosucrase described above. The N-terminal amino acid sequence of the fructosyltransferase purified from *Lactobacillus reuteri* supernatant was found to be QVESNNYNGVAEVNTERQANGQI (residues 2–24 of SEQ ID No. 6). Furthermore, three internal sequences were identified, namely (M)(A)HLDVWDSWPVQDP(V) (SEQ ID No. 7), NAGSIFGT(K) (SEQ ID No. 8), V(E) (E) VYSPKYSTLMASDEVE (SEQ ID No. 9). The N-terminal amino acid sequence could not be identified in the deduced inulosucrase sequence. Also the amino acid sequences of the three internal peptide fragments of the purified fructosyltransferase were not present in the putative inulosucrase sequence. Evidently, the inulosucrase gene does not encode the purified fructosyltransferase synthesizing the levan. The complete amino acid sequence of the levansucrase is shown in SEQ ID No. 11 and the nucleotide sequence is shown in SEQ ID No. 10. The levansucrase comprises a putative membrane anchor (see amino acids 761–765 in SEQ ID No. 11) and a putative membrane spanning domain (see amino acids 766–787 in SEQ ID No. 11). The fructan produced by the levansucrase was identified in the *Lactobacillus reuteri* culture supernatant as a linear (2 \rightarrow 6)- β -D-fructofuranan with a molecular weight of 150 kDa. The purified enzyme also produces this fructan.

Additionally, the invention thus covers a protein having levansucrase activity with an amino acid identity of at least 65%, preferably at least 75%, and more preferably at least 85%, compared to the amino acid sequence of SEQ ID NO. 11. The second novel fructosyltransferase produces a high molecular weight fructan with $\beta(2-6)$ linked fructosyl units with sucrose or raffinose as substrate. The invention also covers a part of a protein with least 15 contiguous amino acids, which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 11. A nucleotide sequence encoding any of the above-mentioned proteins, mutants, variants or parts thereof is a subject of the invention as well as a nucleic acid construct comprising the nucleotide sequence mentioned above operationally linked to an expression-regulating nucleic acid sequence. A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The invention further covers a protein according to the invention which, in the presence of sucrose, produces a fructan having $\beta(2-6)$ -linked D-fructosyl units.

The invention also pertains to a process of producing an inulin-type and/or a levan-type of fructan as described above using fructosyltransferases according to the invention and a

suitable fructose source such as sucrose, stachyose or raffinose. The fructans may either be produced by *Lactobacillus* strains or recombinant host cells according to the invention containing one or both fructosyl transferases or by a fructosyltransferase enzyme isolated by conventional means from the culture of fructosyltransferase-positive *Lactobacilli*, especially a *Lactobacillus reuteri*, or from a recombinant organism containing the fructosyltransferase gene or genes.

Additionally, the invention concerns a process of producing fructo-oligosaccharides containing the characteristic structure of the fructans described above using a *Lactobacillus* strain or a recombinant host cell according to the invention containing one or both fructosyltransferases or an isolated fructosyltransferase according to the invention. There is a growing interest in oligosaccharides derived from homopolysaccharides, for instance for prebiotic purposes. Several fructo- and gluco-oligosaccharides are known to stimulate the growth of bifidobacteria in the human colon. Fructo-oligosaccharides produced by the fructosyltransferase described above are also part of the invention. Another way of producing fructo-oligosaccharides is by hydrolysis of the fructans described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as levanase or inulinase or by acid hydrolysis. The fructo-oligosaccharides can also be produced in the presence of a fructosyltransferase according to the invention and an acceptor molecule such as lactose or maltose. The fructo-oligosaccharides to be produced according to the invention preferably contain at least 2, more preferably at least 3, up to about 20 anhydrofructose units, optionally in addition to one or more other (glucose, galactose, etc.) units. These fructo-oligosaccharides are useful as prebiotics, and can be administered to a mammal in need of improving the bacterial status of the colon.

The invention also concerns chemically modified fructans and fructo-oligosaccharides based on the fructans described above. Chemical modification can be achieved by oxidation, such as hypochlorite oxidation resulting in ring-opened 2,3-dicarboxy-anhydrofructose units (see e.g. EP-A-427349), periodate oxidation resulting in ring-opened 3,4-dialdehyde-anhydrofructose units (see e.g. WO 95/12619), which can be further oxidised to (partly) carboxylated units (see e.g. WO 00/26257), TEMPO-mediated oxidation resulting in 1- or 6-carboxy-anhydrofructose units (see e.g. WO 95/07303). The oxidised fructans have improved water-solubility, altered viscosity and a retarded fermentability and can be used as metal-complexing agents, detergent additives, strengthening additives, bioactive carbohydrates, emulsifiers and water binding agents. They can also be used as starting materials for further derivatisation such as cross-linking and the introduction of hydrophobes. Oxidised fructans coupled to amino compounds such as proteins, or fatty acids can be used as emulsifiers and stabilizers. (Partial) hydrolysis of fructans according to the invention and modified fructans according to the invention results in fructo-oligosaccharides, which can be used as bioactive carbohydrates or prebiotics. The oxidised fructans of the invention preferably contain 0.05–1.0 carboxyl groups per anhydrofructose unit, e.g. as 6- or 1-carboxyl units.

Another type of chemical modification is phosphorylation, as described in O.B. Wurzburg (1986) Modified Starches: properties and uses. CRC Press Inc., Boca Raton, 97–112. One way to achieve this modification is by dry heating fructans with a mixture of monosodium and disodium hydrogen phosphate or with triphosphosphate. The phosphorylated fructans are suitable as wet-end additives in

papermaking, as binders in paper coating compositions, as warp sizing-agents, and as core binders for sand molds for metal casting. A further type of derivatisation of the fructans is acylation, especially acetylation using acetic or propionic anhydride, resulting in products suitable as bleaching assistants and for the use in foils. Acylation with e.g. alkenyl succinic anhydrides or (activated) fatty acids results in surface-active products suitable as e.g. surfactants, emulsifiers, and stabilizers.

Hydroxyalkylation, carboxymethylation, and aminoalkylation are other methods of chemical derivatisation of the fructans. Hydroxyalkylation is commonly performed by base-catalysed reaction with alkylene oxides, such as ethylene oxide, propylene oxide or epichlorohydrine; the hydroxyalkylated products have improved solubility and viscosity characteristics. Carboxymethylation is achieved by reaction of the fructans with mono-chloroacetic acid or its alkali metal salts and results in anionic polymers suitable for various purposes including crystallisation inhibitors, and metal complexants. Amino-alkylation can be achieved by reaction of the fructans with alkylene imines, haloalkyl amines or amino-alkylene oxides, or by reaction of epichlorohydrine adducts of the fructans with suitable amines. These products can be used as cationic polymers in a variety of applications, especially as a wet-end additive in paper making to increase strength, for filler and fines retention, and to improve the drainage rate of paper pulp. Other potential applications include textile sizing and wastewater purification. The above mentioned modifications can be used either separately or in combination depending on the desired product. Furthermore, the degree of chemical modification is variable and depends on the intended use. If necessary 100% modification, i.e. modification of all anhydrofructose units can be performed. However, partial modification, e.g. from 1 modified anhydrofructose unit per 100 up to higher levels, will often be sufficient in order to obtain the desired effect. The modified fructans have a DP (degree of polymerisation) of at least 100, preferably at least 1000 units.

Use of a *Lactobacillus* strain capable of producing a levan, inulin or fructo-oligosaccharides or a mixture thereof, as a probiotic, is also covered by the invention. Preferably, the *Lactobacillus* strain is also capable of producing a glucan, especially an 1,4/1,6- α -glucan as referred to above. The efficacy of some *Lactobacillus reuteri* strains as a probiotics has been demonstrated in various animals such as for instance poultry and humans. The administration of some *Lactobacillus reuteri* strains to pigs resulted in significantly lower serum total and LDL-cholesterol levels, while in children *Lactobacillus reuteri* is used as a therapeutic agent against acute diarrhea. For this and other reasons *Lactobacillus reuteri* strains, which were not reported to produce the glucans or fructans described herein, have been supplemented to commercially available probiotic products. The mode of action of *Lactobacillus reuteri* as a probiotic is still unclear. Preliminary studies indicated that gut colonization by *Lactobacillus reuteri* may be of importance. According to the invention, it was found that the mode of action of *Lactobacillus reuteri* as a probiotic may reside partly in the ability to produce polysaccharides. *Lactobacillus* strains, preferably *Lactobacillus reuteri* strains, and more preferably *Lactobacillus reuteri* strain LB 121 and other strains containing one or more fructosyltransferase genes encoding proteins capable of producing inulins, levans and/or fructo-oligosaccharides can thus advantageously be used as a probiotic. They can also, together with these polysaccharides, be used as a symbiotic (instead of the term symbiotic, the term symbiotic can also be used). In that

respect another part of the invention concerns a probiotic or symbiotic composition containing a *Lactobacillus* strain capable of producing an inulin, a levan or fructo-oligosaccharides and/or a glucan or a mixture thereof, said production being performed according to the process according to the invention. The probiotic or symbiotic compositions of the invention may be directly ingested with or without a suitable vehicle or used as an additive in conjunction with foods. They can be incorporated into a variety of foods and beverages including, but not limited to, yoghurts, ice creams, cheeses, baked products such as bread, biscuits and cakes, dairy and dairy substitute foods, confectionery products, edible oil compositions, spreads, breakfast cereals, juices and the like.

Furthermore, the invention pertains to a process of improving the microbial status in the mammalian colon comprising administering an effective amount of a *Lactobacillus* strain capable of producing an oligosaccharide or polysaccharide according to the invention and to a process of improving the microbial status of the mammalian colon comprising administering an effective amount of an oligosaccharide or polysaccharide produced according to the process according to the invention.

EXAMPLES

EXAMPLE 1

Isolation of DNA from *Lactobacillus reuteri* Nucleotide Sequence Analysis of the Inulosucrase (fitA) Gene, Construction of Plasmids for Expression of the Inulosucrase Gene in *E. coli* Top10 Expression of the Inulosucrase gene in *E. coli* Top10 and Identification of the Produced Polysaccharides Produced by the Recombinant Enzyme.

General procedures for cloning, DNA manipulations and agarose gel electrophoresis were essentially as described by Sambrook et al. (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y. Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the suppliers. DNA was amplified by PCR techniques using ampliTAQ DNA polymerase (Perkin Elmer) or Pwo DNA polymerase. DNA fragments were isolated from agarose gels using the Qiagen extraction kit (Qiagen GmbH), following the instructions of the suppliers. *Lactobacillus reuteri* strain 121 (LMG 18388) was grown at 37° C. in MRS medium (DIFCO) or in MRS-s medium (MRS medium containing 100 g/l sucrose instead of 20 g/l glucose). When fructo-oligosaccharides production was investigated phosphate was omitted and ammonium citrate was replaced by ammonium nitrate in the MRS-s medium. *E. coli* strains were grown aerobically at 37° C. in LB medium, where appropriate supplemented with 50 µg/ml ampicillin (for selection of recombinant plasmids) or with 0.02% (w/v) arabinose (for induction of the inulosucrase gene).

Total DNA of *Lactobacillus reuteri* was isolated according to Verhasselt et al. (1989) FEMS Microbiol. Lett. 59, 135-140 as modified by Nagy et al. (1995) J. Bacteriol. 177, 676-687.

The inulosucrase gene was identified by amplification of chromosomal DNA of *Lactobacillus reuteri* with PCR using degenerated primers (5 fitf, 6 fiti, and 12 fiti, see table 1) based on conserved amino acid sequences deduced from different bacterial fructosyltransferase genes (SacB of *Bacillus amyloliquefaciens*, SacB of *Bacillus subtilis*, *Streptococcus mutans* fructosyltransferase and *Streptococcus salivarius* fructosyltransferase, see FIG. 4) and *Lactobacillus*

reuteri DNA as template. Using primers 5 fitf and 6 fiti, an amplification product with the predicted size of about 234 bp was obtained (FIG. 5A). This 234 bp fragment was cloned in *E. coli* JM109 using the pCR2.1 vector and sequenced. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 µF and 200 Ω, following the instructions of the manufacturer. Sequencing was performed according to the method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467. Analysis of the obtained sequence data confirmed that part of a fructosyltransferase (fitf gene had been isolated. The 234 bp amplified fragment was used to design primers 7 fitf and 8 fiti (see table 1). PCR with the primers 7 fitf and 12 fiti gave a product of the predicted size of 948 bp (see FIG. 5B); its sequence showed clear similarity with previously characterized fructosyltransferase genes. The 948 bp amplified fragment was used to design the primers fitAC1(i) and fitAC2(i) (see table 1) for inverse PCR. Using inverse PCR techniques a 1438 bp fragment of the inulosucrase gene was generated, including the 3' end of the inulosucrase gene (see FIG. 5C). The remaining 5' fragment of the inulosucrase gene was isolated with a combination of standard and inverse PCR techniques. Briefly, *Lactobacillus reuteri* DNA was cut with restriction enzyme XhoI and ligated. PCR with the primers 7 fitf and 8 fiti, using the ligation product as a template, yielded a 290 bp PCR product which was cloned into pCR2.1 and sequenced. This revealed that primer 8 fiti had annealed specifically as well as specifically yielding the 290 bp product (see FIG. 5D).

At this time, the N-terminal amino acid sequence of a fructosyltransferase enzyme (FTFB) purified from the *Lactobacillus reuteri* strain 121 was obtained. This sequence consisted of the following 23 amino acids: QVESNNYN-GVAEVNTERQANGQI (residues 2-24 of SEQ ID No. 6). The degenerated primer 19 fitf (YNGVAEV) (residues 8-14 of SEQ ID NO: 6) was designed on the basis of a part of this N-terminal peptide sequence and primer 20 fiti was designed on the 290 bp PCR product. PCR with primers 19 fitf and 20 fiti gave a 754 bp PCR product (see FIG. 5E), which was cloned into pCR2.1 and sequenced. Both DNA strands of the entire fructosyltransferase gene were double sequenced. In this way the sequence of a 2.6 kb region of the *Lactobacillus reuteri* DNA, containing the inulosucrase gene and its surroundings were obtained.

The plasmids for expression of the inulosucrase gene in *E. coli* Top10 were constructed as described hereafter. A 2414 bp fragment, containing the inulosucrase gene starting at the first putative start codon at position 41, was generated by PCR, using primers fitA1 and fitA2i. Both primers contained suitable restriction enzyme recognition sites (a NcoI site at the 5' end of fitA1 and a BglII site at the 3' end of fitA2i). PCR with *Lactobacillus reuteri* DNA, Pwo DNA polymerase and primers fitA1 and fitA2i yielded the complete inulosucrase gene flanked by NcoI and BglII restriction sites. The PCR product with blunt ends was ligated directly into pCRblunt1-Topo. Using the NcoI and BglII restriction sites, the putative fitA gene was cloned into the expression vector pBAD, downstream of the inducible arabinose promoter and in frame upstream of the Myc epitope and the His tag. The pBAD vector containing the inulosucrase gene (pSVH101) was transformed to *E. coli* Top10 and used to study inulosucrase expression. Correct construction of plasmid containing the complete inulosucrase gene was confirmed by restriction enzyme digestion analysis and by sequence analysis, showing an in frame cloning of the inulosucrase gene using the ribosomal binding site provided by the pBAD vector and the first putative start codon (at position 41) of inulosucrase (see FIG. 1).

Plasmid DNA of *E. coli* was isolated using the alkaline lysis method of Birnboim and Doly (1979) *Nucleic Acids Res.* 7, 1513-1523 or with a Qiagen plasmid kit following the instructions of the supplier. Cells of *E. coli* Top10 with pSVH101 were grown overnight in LB medium containing 0.02% (w/v) arabinose and were harvested by centrifugation. The pellet was washed with 25 mM sodium acetate buffer pH 5.4 and the suspension was centrifuged again. Pelleted cells were resuspended in 25 mM sodium acetate buffer pH 5.4. Cells were broken by sonication. Cell debris and intact cells were removed by centrifugation for 30 min at 4° C. at 10,000×g and the resulting cell free extract was used in the enzyme assays.

The fructosyltransferase activities were determined at 37° C. in reaction buffer (25 mM sodium acetate, pH 5.4, 1 mM CaCl₂, 100 g/l sucrose) by monitoring the release of glucose from sucrose, by detecting fructo-oligosaccharides or by determining the amount of fructan polymer produced using *E. coli* cell free extracts or *Lactobacillus reuteri* culture supernatant as enzyme source. Sucrose, glucose and fructose were determined enzymatically using commercially available kits.

Fructan production by *Lactobacillus reuteri* was studied with cells grown in MRS-s medium. Product formation was also studied with cell-free extracts of *E. coli* containing the novel inulosucrase incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37° C.). Fructans were collected by precipitation with ethanol. ¹H-NMR spectroscopy and methylation analysis were performed as described by van Geel-Schutten et al. (1999) *Appl. Environ. Microbiol.* 65, 3008-3014. The molecular weights of the fructans were determined by high performance size exclusion chromatography coupled on-line with a multi angle laser light scattering and a differential refractive index detector. Fructo-oligosaccharide synthesis was studied in *Lactobacillus reuteri* culture supernatants and in extracts of *E. coli* cells containing the novel inulosucrase incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37° C.). Glucose and fructose were determined enzymatically as described above and fructo-oligosaccharides produced were analyzed using a Dionex column. The incubation mixtures were centrifuged for 30 min at 10,000×g and diluted 1:5 in a 100% DMSO solution prior to injection on a Dionex column. A digest of inulin (DPI-20) was used as a standard. Separation of compounds was achieved with anion-exchange chromatography on a CarboPac Pa1 column (Dionex) coupled to a CarboPac PA1 guard column (Dionex). Using a Dionex GP50 pump the following gradient was generated: % eluent B is 5% (0 min); 35% (10 min); 45% (20 min); 65% (50 min); 100% (54-60 min); 5% (61-65 min). Eluent A was 0.1 M NaOH and eluent B was 0.6 M NaAc in a 0.1 M NaOH solution. Compounds were detected using a Dionex ED40 electrochemical detector with an AU working electrode and a Ag/AgCl reference-electrode with a sensitivity of 300 nC. The pulse program used was: +0.1 Volt (0-0.4 s); +0.7 Volt (0.41-0.60 s); -0.1 Volt (0.61-1.00 s). Data were integrated using a Perkin Elmer Turbochrom data integration system. A different separation of compounds was done on a cation exchange column in the calcium form (Benson BCX4). As mobile phase Ca-EDTA in water (100 ppm) was used. The elution speed was 0.4 ml/min at a column temperature of 85° C. Detection of compounds was done by a refractive index (Jasco 830-RI) at 40° C. Quantification of compounds was achieved by using the software program Turbochrom (Perkin Elmer).

SDS-PAGE was performed according to Laemmli (1970) *Nature* 227, 680-685 using 7.5% polyacrylamide gels. After

electrophoresis gels were stained with Coomassie Brilliant Blue or an activity staining (Periodic Acid Schiff, PAS) was carried out as described by Van Geel-Schutten et al. (1999) *Appl. Environ. Microbiol.* 65, 3008-3014.

TABLE 1

Nucleotide sequence of primers used in PCR reactions to identify the inulosucrase gene.

Primer name	Location (bp)	Nucleotide sequence (and SEQ ID No)
rfAC1	1176	CTG-ATA-ATA-ATG-GAA-ATG-TAT-CAC (SEQ ID No. 12)
rfAC2i	1243	CAT-GAT-CAT-AAG-TTT-GGT-AGT-AAT-AG (SEQ ID No. 13)
rfAC1	1176	GTG-ATA-CAT-TTC-CAT-TAT-TAT-CAG (SEQ ID No. 14)
rfAC2	1243	CTA-TTA-CTA-CCA-AAC-TTA-TGA-TCA-TG (SEQ ID No. 15)
rfA1		CCA-TGG-CCA-TGG-TAG-AAC-GCA-AGG- AAC-ATA-AAA-AAA-TG (SEQ ID No. 16)
rfA2i		AGA-TCT-AGA-TCT-GTT-AAA-TCG-ACG-TTT- GTT-AAT-TTC-TG (SEQ ID No. 17)
5rf	845	GAY-GTN-TGG-GAY-WSN-TGG-GCC (SEQ ID No. 18)
6rf	1052	GTN-GCN-SWN-CCN-SWC-CAY-TSY-TG (SEQ ID No. 19)
7rf	1009	GAA-TGT-AGG-TCC-AAT-TTF-TGG-C (SEQ ID No. 20)
8rf	864	CCT-GTC-CGA-ACA-TCT-TGA-ACT-G (SEQ ID No. 21)
12rf	1934	ARR-AAN-SWN-GCN-GCV-MAN-GTN-SW (SEQ ID No. 22)
19rf	1	TAY-AYY-GGN-GTN-GCN-GAR-GTN-AA (SEQ ID No. 23)
20rf	733	CCG-ACC-ATC-TTG-TTT-GAT-TAA-C (SEQ ID No. 24)

Listed from left to right are: primer name (i, inverse primer), location (in bp) in fTA and the sequence from 5' to 3' according to IUB group codes (N = any base; M = A or C; R = A or G; W = A or T; S = C or G; Y = C or T; K = G or T; B = not A; D = not C; H = not G; and V = not T).

EXAMPLE 2

Purification and Amino Acid Sequencing of the Levansucrase (FTFB).

Protein Purification

Samples were taken between each step of the purification process to determine the enzyme activity (by glucose GOD-Perid method) and protein content (by Bradford analysis and acrylamide gel electrophoresis). Collected chromatography fractions were screened for glucose liberating activity (GOD-Perid method) to determine the enzyme activity.

One liter of an overnight culture of LB121 cells grown on MRS medium containing 50 grams per liter maltose was centrifuged for 15 min. at 10,000×g. The supernatant was precipitated with 1.5 liter of a saturated ammonium sulphate solution. The ammonium sulphate solution was added at a rate of 50 ml/min. under continuous stirring. The resulting 60% (w/v) ammonium sulphate solution was centrifuged for 15 min. at 10,000×g. The precipitate was resuspended in 10 ml of a sodium phosphate solution (10 mM, pH 6.0) and dialysed overnight against 10 mM sodium phosphate, pH 6.0.

A hydroxylapatite column was washed with a 10 mM sodium phosphate solution pH 6.0; the dialysed sample was loaded on the column. After eluting the column with 200 mM sodium phosphate, pH 6.0 the eluted fractions were screened for glucose releasing activity and fractions were pooled for phenyl superose (a hydrophobic interactions column) chromatography. The pooled fractions were diluted

1:1 (v:v) with 25 mM sodium acetate, 2 M ammonium sulphate, pH 5.4 and loaded on a phenyl superose column (washed with 25 mM sodium acetate, 1 M ammonium sulphate, pH 5.4). In a gradient from 25 mM sodium acetate, 1 M ammonium sulphate, pH 5.4 (A) to 25 mM sodium acetate, pH 5.4 (B) fractions were collected from 35% B to 50% B.

Pooled fractions from the phenyl superose column were loaded on a gel filtration (superdex) column and eluted by a 25 mM acetate, 0.1 M sodium chloride, pH 5.4 buffer. The superdex fractions were loaded on a washed (with 25 mM sodium acetate, pH 5.4) Mono Q column and eluted with 25 mM sodium acetate, 1 M sodium chloride, pH 5.4. The fractions containing glucose liberating activity were pooled, dialysed against 25 mM sodium acetate, pH 5.4, and stored at -20° C.

A levansucrase enzyme was purified from LB121 cultures grown on media containing maltose using ammonium sulfate precipitation and several chromatography column steps (table 2). Maltose (glucose—glucose) was chosen because both glucanase and levansucrase can not use maltose as substrate. LB121 will grow on media containing maltose but will not produce polysaccharide. From earlier experiments it was clear that even with harsh methods the levansucrase enzyme could not be separated from its product levan. These harsh methods included boiling the levan in a SDS solution and treating the levan with HCl and TFA. No levansucrase enzyme was commercially available for the enzymatic breakdown of levan. Only a single levansucrase was detected in maltose culture supernatants. In order to prove that the enzyme purified from maltose culture supernatant is the same enzyme which is responsible for the levan production during growth on raffinose, biochemical and biophysical tests were performed.

TABLE 2

Purification of the <i>Lactobacillus reuteri</i> LB 121 levansucrase (FTFB) enzyme					
Step	Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Purification (fold)	Yield (%)
Supernatant	128	64	0.5	1	100
Ammonium sulfate precipitation (65%)	35.2	42	1.2	2.4	65.6
Hydroxyl apatite	1.5	30.6	20.4	40.8	47.8
Phenyl superose	0.27	23	85	170	36
Gel Filtration	0.055	10	182	360	16
MonoQ	0.0255	4	176	352	6

Amino Acid Sequencing of FTFB

A 5% SDS-PAA gel was allowed to "age" overnight in order to reduce the amount of reacting chemical groups in the gel. Reaction of chemicals in the PAA gel (TEMED and ammonium persulfate) with proteins can cause some undesired effects, such as N-terminal blocking of the protein, making it more difficult to determine the protein amino acid composition. 0.1 mM thioglycolic acid (scavenger to reduce the amount of reactive groups in the PAA gel material) was added to the running buffer during electrophoresis.

In order to determine the amino acid sequence of internal peptides of protein bands running in a SDS-PAA gel, protein containing bands were cut out of the PAA gel. After frac-

tionating the protein by digestion with chymotrypsin the N-terminal amino acid sequences of the digested proteins were determined (below).

N-terminal sequencing was performed by Western blotting of the proteins from the PAA gel to an Immobilon PVDF membrane (Millipore/Waters Inc.) at 0.8 mA/cm² for 1 h. After staining the PVDF membrane with Coomassie Brilliant Blue without adding acetic acid (to reduce N-terminal blocking) and destaining with 50% methanol, the corresponding bands were cut out of the PVDF membrane for N-terminal amino acid sequence determination.

Amino acid sequence determination was performed by automated Edman degradation as described by Koningsberg and Steinman (1977) The proteins (third edition) volume 3, 1-178 (Neurath and Hill, eds.). The automated equipment for Edman degradation was an Applied Biosystems model 477A pulse-liquid sequencer described by Hewick et al. (1981), J. Biol. Chem. 15, 7990-7997 connected to a RP-HPLC unit (model 120A, Applied Biosystems) for amino acid identification.

The N-terminal sequence of the purified FTFB was determined and found to be: (A) Q V E S N N Y N G V A E V N T E R Q A N G Q I (G) (V) (D) (SEQ ID No. 6). Three internal peptide sequences of the purified FTFB were determined: (M) (A) H L D V W D S W P V Q D P (V) (SEQ ID No. 7); N A G S I F G T (K) (SEQ ID No. 8); and V (E) (E) V Y S P K V S T L M A S D E V E (SEQ ID No. 9).

The following primers were designed on the basis of the N-terminal and internal peptide fragments of FTFB. Listed from left to right are: primer name, source peptide fragment and sequence (from 5' to 3'). FTFB1+FTFB3i yields approximately a 1400 bp product in a PCR reaction. FTFB1 forward (N-terminal): AA T/C-TAT-AA T/C-GG T/C-GTT-GC G/A-T/C GA-AGT (SEQ ID No. 25); and FTFB3i reverse (Internal 3): TAC-CGN-A/T C/G N-CTA-CTT-CAA-CTT (SEQ ID No. 26). The FTFB gene was partly isolated by PCR with primers FTFB1 and FTFB3i. PCR with these primers yielded a 1385 bp amplicon, which after sequencing showed high homology to ftfA and SacB from *Streptococcus mutans*.

EXAMPLE 3

Oxidation of Levans

For TEMPO-mediated oxidation, a levan according to the invention prepared as described above (dry weight 1 g, 6.15 mmol) was resuspended in 100 ml water. Next, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO; 1% by weight compared to the polysaccharide (0.01 g, 0.065 mmol)) was added and resuspended in 20 min. Sodium bromide (0.75 g, 7.3 mmol) was added and the suspension was cooled down to 0° C. This reaction also proceeded without bromide. A solution of hypochlorite (6 ml, 15% solution, 12.6 mmol) was adjusted to pH 10.0 with 3M HCl and cooled to 0° C. This solution was added to the suspension of the polysaccharide and TEMPO. The course of the reaction was followed by monitoring the consumption of sodium hydroxide solution, which is equivalent to the formation of uronic acid. After 30 min, 60 ml 0.1M NaOH was consumed. This amount corresponds to the formation of 97% uronic acid. Thereafter, the solution was poured out in 96% ethanol (comprising 70% of the volume of the solution) causing the product to precipitate. The white precipitate was centrifuged, resuspended in ethanol/water (70/30 v/v) and centrifuged again. Next, the precipitate was resuspended in 96% ethanol and centrifuged. The obtained product was dried at reduced pressure. The uronic acid content was determined by means of the uronic acid assay according to Blumenkrantz and Abdoe-Hansen (Anal. Biochem., 54

(1973), 484). A calibration curve was generated using polygalacturonic acid (5, 10, 15 and 20 μg). With this calibration curve the uronic acid content in a sample of 20 μg of the product was determined. The obtained result was a content of 95% uronic acid with a yield of 96%.

Partial Oxidation

For partial oxidation, a levan according to the invention (dry weight 2 g, 12.3 mmol) was resuspended in 25 ml water. Next, TEMPO (1% by weight compared to the polysaccharide (0.02 g, 0.13 mmol)) was added, resuspended in 20 min and cooled to 0° C. A solution of hypochlorite (1 ml, 15% solution, 2.1 mmol) was adjusted to pH 9.0 with 3M HCl and cooled down to 0° C. This solution was added to the suspension of the polysaccharide and TEMPO. Within 5 min the mixture became a solid gel.

EXAMPLE 4

Adhesion of *Lactobacillus reuteri* Strains to Caco-2 Cell Lines

The adhesion of *Lactobacillus reuteri* strains to Caco-2 cell lines was determined as described below. Firstly, a bacterial suspension was prepared as follows. *Lactobacillus reuteri* strains LB 121, 35-5, K24 and DSM20016 and *L. rhamnosus* LGG (a well known probiotic strain with good adhering properties) were cultured in MRS broth supplemented with 5 $\mu\text{l/ml}$ of methyl-1,2- ^3H -thymidine at 37° C. for 18–20 h before the adhesion assays. The cultures were harvested by centrifugation, washed with phosphate buffered saline (PBS) and resuspended in PBS or PBS supplemented with 30 g/l sucrose (see Table 3) to a final density of about 2×10^9 cfu/ml. Prior to the adhesion assay, the cell suspensions in PBS with 30 g/l sucrose were incubated for 1 hour at 37° C., whereas the cell suspensions in PBS were kept on ice for 1 hour. After incubation at 37° C., the suspensions in PBS with sucrose were centrifuged and the cells were washed with and resuspended in PBS to a final density of about 2×10^9 cfu/ml.

Caco-2 cells were cultured as follows. Subcultures of Caco-2 cells (ATCC, code HTB 37, human colon adenocarcinoma), stored as frozen stock cultures in liquid nitrogen were used for the adhesion tests. The Caco-2 cells were grown in culture medium consisting of Dulbecco's modified Eagle medium (DMEM), supplemented with heat-inactivated foetal calf serum (10% v/v), non-essential amino acids (1% v/v), L-glutamine (2 mM) and gentamicin (50 $\mu\text{g/ml}$). About 2,000,000 cells were seeded in 75 cm^2 tissue culture flasks containing culture medium and cultured in a humidified incubator at 37° C. in air containing 5% CO_2 . Near confluent Caco-2 cell cultures were harvested by trypsinisation and resuspended in culture medium. The number of cells was established using a Bürker-Türk counting chamber.

TABLE 3

Incubation of the different <i>Lactobacillus</i> strains prior to the adhesion assays.			
Lactobacillus strain	Extra incubation	Polysaccharide produced	Group
reuteri 121	PBS sucrose, 37° C. for 1 hr	glucan and fructan	As
reuteri 35-5	PBS sucrose, 37° C. for 1 hr	glucan	Bs
reuteri K24	PBS sucrose, 37° C. for 1 hr	none	Cs

TABLE 3-continued

Incubation of the different <i>Lactobacillus</i> strains prior to the adhesion assays.			
Lactobacillus strain	Extra incubation	Polysaccharide produced	Group
reuteri 121	PBS on ice	none	D
reuteri	PBS on ice	none	E
DSM20016*			
rhamnosus GG	PBS on ice	none	F

*Type strain of *L. reuteri*

For the following experiments a Caco-2 monolayer transport system was used. Caco-2 cells cultured in a two-compartment transport system are commonly used to study the intestinal, epithelial permeability. In this system the Caco-2 cell differentiates into polarized columnar cells after reaching confluency. The Caco-2 system has been shown to simulate the passive and active transcellular transport of electrolytes, sugars, amino acids and lipophilic compounds (Hillgren et al. 1995, Duffer et al., 1996, Duizer et al., 1997). Also, a clear correlation between the in vivo absorption and the permeability across the monolayers of Caco-2 cells has been reported (Artursson and Karlsson, 1990). For the present transport studies, Caco-2 cells were seeded on semi-permeable filter inserts (12 wells Transwell plates, Costar) at ca. 100,000 cells per filter (growth area $\pm 1 \text{ cm}^2$ containing 2.5 ml culture medium). The cells on the insert were cultured for 17 to 24 days at 37° C. in a humidified incubator containing 5% CO_2 in air. During this culture period the cells have been subjected to an enterocyte-like differentiation. Gentamycin was eliminated from the culture medium two days prior to the adhesion assays.

The adhesion assay was performed as follows. PBS was used as exposure medium. 25 μl of a bacterial suspension (2×10^9 cfu/ml) were added to 0.5 ml medium. The apical side of the Caco-2 monolayers was incubated with the bacterial suspensions for 1 hour at 37° C. After incubation, remaining fluid was removed and the cells were washed three times with 1 ml PBS. Subsequently, the Caco-2 monolayers were digested overnight with 1 ml 0.1M NaOH, 1% SDS. The lysate was mixed with 10 ml Hionic Fluor scintillation liquid and the radioactivity was measured by liquid scintillation counting using a LKB/Wallac scintillation counter. As a control, the radioactivity of the bacterial suspensions was measured. For each test group, the percentage of bacteria attached to the monolayers was calculated. All adhesion tests were performed in quadruplicate. In Table 4 the results of the bacterial adhesion test to Caco-2 cell lines are given. From the results can be concluded that the glucans and the fructans contribute to the adherence of *Lactobacillus reuteri* to Caco-2 cell lines. This could indicate that *Lactobacillus reuteri* strains producing EPS possess improved probiotic characteristics or that *Lactobacillus reuteri* and its polysaccharides could function as an excellent symbiotic.

TABLE 4

The results of the bacterial adhesion test to Caco-2 cell lines.		
Group (see Table 1)	0% of bacteria bound to the monolayer	
As	6.5	
Bs	5.7	
Cs	1.8	

TABLE 4-continued

The results of the bacterial adhesion test to Caco-2 cellines.	
Group (see Table 1)	% of bacteria bound to the monolayer
D	2.3
E	0.9
F	1.3

DESCRIPTION OF THE FIGURES

FIG. 1: The nucleic acid (SEQ ID NO: 4) and deduced amino acid sequences (SEQ ID NOS 27 and 1) of the novel inulosucrase of *Lactobacillus reuteri*. Also encompassed within the figure is the comparison peptide (SEQ ID NO: 28). Furthermore, the designations and orientation (< for 3' to 5' and > for 5' to 3') of the primers and the restriction enzymes used for (inverse) PCR, are shown at the right hand side. Putative start codons (ATG, at positions 41 and 68) and stop codon (TAA, at position 2435) are shown in bold. The positions of the primers used for PCR are shown in bold/underlined. The NheI restriction sites (at positions 1154 and 2592) used for inverse PCR are underlined. The primers used and their exact positions in the inulosucrase sequence are shown in table 1. Starting at amino acid 690, the 20 PXX (residues 690-749 of SEQ ID NO: 1) repeats are underlined. At amino acid 755 the LPXTG (SEQ ID NO: 5) motif is underlined.

FIG. 2: Dendrogram of bacterial and plant fructosyltransferases. The horizontal distances are a measure for the

difference at the amino acid sequence level. 10% difference is indicated by the upper bar. Bootstrap values (in percentages) are given at the root of each tree. Fructosyltransferases of Gram positive bacteria are indicated in the lower half of the figure (*B. stercorophilus* SurB; *B. amyloliquefaciens* SacB; *B. subtilis* SacB; *S. mutans* SacB; *L. reuteri* FtfA (inulosucrase); *S. salivarius* Ftf). Plant fructosyltransferases are indicated in the middle part of the figure (*Cynara scolymus* Ss-1 ft; *Allium cepa* F-6 gft; *Hordeum vulgare* Sf-6 ft). Fructosyltransferases of Gram negative bacteria are shown in the upper part of the figure (*Z. mobilis* LevU; *Z. mobilis* SucE2; *Z. mobilis* SacB; *E. amylovora* Lcs; *A. diazotrophicus* LsdA).

FIG. 3: The N-terminal (SEQ ID NO: 6) and three internal amino acid sequences (SEQ ID NOS 7-9) of the novel levansucrase of *Lactobacillus reuteri*.

FIG. 4: Parts of an alignment of the deduced amino acid sequences of some bacterial fructosyltransferase genes (SEQ ID NOS 29-40). Sequences in bold indicate the consensus sequences used to construct the degenerated primers 5 ftf, 6 ftf and 12 ftf. (*) indicates a position with a fully conserved amino acid residue. (.) indicates a position with a fully conserved 'strong' group: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW. (.) indicates a position with a fully conserved 'weaker' group: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, HFY. Groups are according to the Pam250 residue weight matrix described by Altschul et al. (1990) J. Mol. Biol. 215, 403-410.

FIG. 5: The strategy used for the isolation of the inulosucrase gene from *Lactobacillus reuteri* 121 chromosomal DNA.

SEQUENCE LISTING

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Ile Glu Asn Asn Asp Ser Ser Thr Val Gln Val Thr Thr Gly Asp Asn
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 50             55             60

Ala Ser Asp Thr Thr Ile Arg Thr Ser Ala Asn Ala Asn Ser Ala Ser
 65             70             75             80

Ser Ala Ala Asn Thr Gln Asn Ser Asn Ser Gln Val Ala Ser Ser Ala
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Ala Ile Thr Ser Ser Thr Ser Ser Ala Ala Ser Leu Asn Asn Thr Asp
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Glu Pro Leu Asp Val Trp Asp Ser Trp Pro Val Gln Asp Val Arg Thr 260 265 270		
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 660 665 670
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	285				290				295					300		
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Val	Ile	Ala	Met	Met	Gly	Ile	Pro	Asn	Gln	Asn	Asp	Asn	His	Ile	Tyr	
		305					310						315			

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ccc tta tat aat aag tat ggt gat aat gaa tta agt cat tgg aag aat Leu Leu Tyr Aen Lys Tyr Gly Asp Aen Glu Leu Ser His Trp Lys Aen 320 325 330	1012
gta ggt cca att ttt ggc tat aat tct acc gcg gtt tca caa gaa tgg Val Gly Pro Ile Phe Gly Tyr Aen Ser Thr Ala Val Ser Gln Glu Trp 335 340 345	1060
tca gga tca gct gtt ttg aac agt gat aac tct atc caa tta ttt tat Ser Gly Ser Ala Val Leu Aen Ser Asp Aen Ser Ile Gln Leu Phe Tyr 350 355 360	1108
aca agg gta gac acg tct gat aac aat acc aat cat caa aaa att gct Thr Arg Val Asp Thr Ser Asp Aen Aen Thr Aen His Gln Lys Ile Ala 365 370 375 380	1156
agc gct act ctt tat tta act gat aat aat gga aat gta tca ctc gct Ser Ala Thr Leu Tyr Leu Thr Asp Aen Aen Gly Aen Val Ser Leu Ala 385 390 395	1204
cag gta cga aat gac tat att gta ttt gaa ggt gat ggc tat tac tac Gln Val Arg Aen Aep Tyr Ile Val Phe Glu Gly Asp Gly Tyr Tyr Tyr 400 405 410	1252
caa act tat gat caa tgg aaa gct act aac aaa ggt gcc gat aat att Gln Thr Tyr Asp Gln Trp Lys Ala Thr Aen Lys Gly Ala Asp Aen Ile 415 420 425	1300
gca atg cgt gat gct cat gta att gaa gat ggt aat ggt gat cgg tac Ala Met Arg Asp Ala His Val Ile Glu Asp Gly Aen Gly Asp Arg Tyr 430 435 440	1348
ctt gtt ttt gaa gca agt act ggt ttg gaa aat tat caa ggc gag gac Leu Val Phe Glu Ala Ser Thr Gly Leu Glu Aen Tyr Gln Gly Glu Asp 445 450 455 460	1396
caa att tat aac tgg tta aat tat ggc gga gat gac gca ttt aat atc Gln Ile Tyr Aen Trp Leu Aen Tyr Gly Gly Asp Asp Ala Phe Aen Ile 465 470 475	1444
aag agc tta ttt aga att ctt tcc aat gat gat att aag agt cgg gca Lys Ser Leu Phe Arg Ile Leu Ser Aen Asp Asp Ile Lys Ser Arg Ala 480 485 490	1492
act tgg gct aat gca gct atc ggt atc ctc aaa cta aat aag gac gaa Thr Trp Ala Aen Ala Ala Ile Gly Ile Leu Lys Leu Aen Lys Asp Glu 495 500 505	1540
aag aat cct aag gtg gca gag tta tac tca cca tta att tct gca cca Lys Aen Pro Lys Val Ala Glu Leu Tyr Ser Pro Leu Ile Ser Ala Pro 510 515 520	1588
atg gta agc gat gaa att gag cga cca aat gta gtt aaa tta ggt aat Met Val Ser Asp Glu Ile Glu Arg Pro Aen Val Val Lys Leu Gly Aen 525 530 535 540	1636
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gtc gga tat gtt gct gat agt cta act gga tct tat aag cca tta aat Val Gly Tyr Val Ala Asp Ser Leu Thr Gly Ser Tyr Lys Pro Leu Aen 575 580 585	1780
gat tct gga gta gtc ttg act gct tct gtt cct gca aac tgg cgg aca Asp Ser Gly Val Val Leu Thr Ala Ser Val Pro Ala Aen Trp Arg Thr 590 595 600	1828
gca act tat tca tat tat gct gtc ccc gtt gcc gga aaa gat gac caa Ala Thr Tyr Ser Tyr Ala Val Pro Val Ala Gly Lys Asp Asp Gln 605 610 615 620	1876
gta tta gtt act tca tat atg act aat aga aat gga gta cgg ggt aaa Val Leu Val Thr Ser Tyr Met Thr Aen Arg Aen Gly Val Ala Gly Lys 625 630 635	1924

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gga atg gat tca act tgg gca cgg agt ttc tta cta caa att aac ccg 1972
Gly Met Asp Ser Thr Trp Ala Pro Ser Phe Leu Leu Gln Ile Asn Pro
      640      645      650

gat aac aca act act gtt tta gct aaa atg act aat caa ggg gat tgg 2020
Asp Asn Thr Thr Thr Val Leu Ala Lys Met Thr Asn Gln Gly Asp Trp
      655      660      665

att tgg gat gat tca agc gaa aat ctt gat atg att ggt gat tta gac 2068
Ile Trp Asp Asp Ser Ser Glu Asn Leu Asp Met Ile Gly Asp Leu Asp
      670      675      680

tcc gct gct tta cct ggc gaa cgt gat aaa cct gtt gat tgg gac tta 2116
Ser Ala Ala Leu Pro Gly Glu Arg Asp Lys Pro Val Asp Trp Asp Leu
      685      690      695

att ggt tat gga tta aaa ccg cat gat cct gct aca cca aat gat cct 2164
Ile Gly Tyr Gly Leu Lys Pro His Asp Pro Ala Thr Pro Asn Asp Pro
      705      710      715

gaa acg cca act aca cca gaa acc cct gag aca cct aat act ccc aaa 2212
Glu Thr Pro Thr Thr Pro Glu Thr Pro Glu Thr Pro Asn Thr Pro Lys
      720      725      730

aca cca aag act cct gaa aat cct ggg aca cct caa act cct aat aca 2260
Thr Pro Lys Thr Pro Glu Asn Pro Gly Thr Pro Gln Thr Pro Asn Thr
      735      740      745

cct aat act ccg gaa att cct tta act cca gaa acg cct aag caa cct 2308
Pro Asn Thr Pro Glu Ile Pro Leu Thr Pro Glu Thr Pro Lys Gln Pro
      750      755      760

gaa acc caa act aat aat cgt ttg cca caa act gga aat aat gcc aat 2356
Glu Thr Gln Thr Asn Asn Arg Leu Pro Gln Thr Gly Asn Asn Ala Asn
      765      770      775      780

aaa gcc atg att ggc cta ggt atg gga aca ttg ctt agt atg ttt ggt 2404
Lys Ala Met Ile Gly Leu Gly Met Gly Thr Leu Leu Ser Met Phe Gly
      785      790      795

ctt gca gaa att aac aaa cgt cga ttt aac taatactttt aaataaaac 2454
Leu Ala Glu Ile Asn Lys Arg Arg Phe Asn
      800      805

cgctaagcct taattcagc ttaacggttt tttattttaa aagtttttat tgtaaaaaag 2514

cgaattatca ttaatactaa tgcaattggt gtaagacctt acgacagtag taacaatgaa 2574

tttgcccatc tttgtcgg 2592

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<210> SEQ ID NO 5
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<212> TYPE: PRT
<213> ORGANISM: Lactobacillus reuteri
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (3)
<223> OTHER INFORMATION: Any amino acid

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<400> SEQUENCE: 5

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Leu Pro Xaa Thr Gly
1          5

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<210> SEQ ID NO 6
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus reuteri

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<400> SEQUENCE: 6

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Ala Gln Val Glu Ser Asn Asn Tyr Asn Gly Val Ala Glu Val Asn Thr
1          5          10          15

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Glu Arg Gln Ala Asn Gly Gln Ile Gly Val Asp
20          25

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<210> SEQ ID NO 7
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Lactobacillus reuteri

<400> SEQUENCE: 7

Met Ala His Leu Asp Val Trp Asp Ser Trp Pro Val Gln Asp Pro Val
 1 5 10 15

<210> SEQ ID NO 8
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Lactobacillus reuteri

<400> SEQUENCE: 8

Asn Ala Gly Ser Ile Phe Gly Thr Lys
 1 5

<210> SEQ ID NO 9
 <211> LENGTH: 19
 <212> TYPE: PRT
 <213> ORGANISM: Lactobacillus reuteri

<400> SEQUENCE: 9

Val Glu Glu Val Tyr Ser Pro Lys Val Ser Thr Leu Met Ala Ser Asp
 1 5 10 15

Glu Val Glu

<210> SEQ ID NO 10
 <211> LENGTH: 4634
 <212> TYPE: DNA
 <213> ORGANISM: Lactobacillus reuteri
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1220)..(3598)
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 <222> LOCATION: (1205)..(1210)
 <220> FEATURE:
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 <222> LOCATION: (2702)..(2707)
 <223> OTHER INFORMATION: a, c, t, g, other or unknown
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 <222> LOCATION: (3686)..(3698)
 <223> OTHER INFORMATION: a, c, t, g, other or unknown

<400> SEQUENCE: 10

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 aatgtgccta ggaatcataa tggatgtaaa ttactagatg gcggttttta tacattaacc 180
 tcgcaggaga gaaaagaagc aattagtaag gatccatag cagataaatt tattaggcct 240
 tatttaggtg ctaaaaattt cattcatgga actgctaggt actgtatttg gttaaaggac 300
 gcaaacccga aagatatcca tcaatcgcca ttatactgg atagaatcaa taagtagcg 360
 gaattcagat cgcagcaaaa aagtaagat acacaaaaat atgcaaaacg gcccatgcta 420
 acaacacgac ttgcctatta tagccacgat gtacatacgg atagtctgat agtacctgca 480
 acatcatcgc aacgtagaga atatcttcca attggatatg ttccagaaaa gaattatttg 540
 tcttattcac taatgcta atccccatgct agtaatttta atttcggtat tctagaatct 600
 aaagttcact atatttggtt aaaaaacttt tgcggtcggt tgaagtcgca ttatcgttat 660

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tcaaacacta ttatttataa taatttcctt tggccgactg ttggtgacaa gccaggamca	720
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tcactggctg atctttatga tccactaaca atgccragtt gaactcgtaa agctcatgaa	840
gccaatgata aagctgttct taagcatat ggattgagcc ctaagctac tgagcaagaa	900
atcgtagaa acctatttaa gatgtatgaa aaactgacta aaggtgaaag ataactttgt	960
aaaaccaata ttttataaag acagtaaatg ttaatttgat aaaaacatat atttaataaa	1020
caaaagtgat ataataaagt agttctttgt attacaaaa acatttaata tctctcagca	1080
ttttgcatac tgggagattt tttattgaca aattgtttga aagtgcttat gatgaacag	1140
tgtagaaact aattcaattt gataaacgtt agacatttct gaggaggaag tcattttgga	1200
gtacaaagaa cataagaaa atg tat aaa gtc gcc aag aat tgg gcc gtt gct	1252
Met Tyr Lys Val Gly Lys Asn Trp Ala Val Ala	
1 5 10	
aca ttg gta tca gct tca att tta atg gga ggg gtt gta acc gct cat	1300
Thr Leu Val Ser Ala Ser Ile Leu Met Gly Gly Val Val Thr Ala His	
15 20 25	
gct gat caa gta gaa agt aac aat tac aac ggt gtt gct gaa gtt aat	1348
Ala Asp Gln Val Glu Ser Asn Asn Tyr Asn Gly Val Ala Glu Val Asn	
30 35 40	
act gaa cgt caa gct aat ggt caa att ggc gta gat gga aaa att att	1396
Thr Glu Arg Gln Ala Asn Gly Gln Ile Gly Val Asp Gly Lys Ile Ile	
45 50 55	
agt gct aac agt aat aca acc agt ggc tcg aca aat caa gaa tca tct	1444
Ser Ala Asn Ser Asn Thr Thr Ser Gly Ser Thr Asn Gln Glu Ser Ser	
60 65 70 75	
gct act aac aat act gaa aat gct gtt gtt aat gaa agc aaa aat act	1492
Ala Thr Asn Asn Thr Glu Asn Ala Val Val Asn Glu Ser Lys Asn Thr	
80 85 90	
aac aat act gaa aat gct gtt gtt aat gaa aac aaa aat act aac aat	1540
Asn Asn Thr Glu Asn Ala Val Val Asn Glu Asn Lys Asn Thr Asn Asn	
95 100 105	
act gaa aat gct gtt gtt aat gaa aac aaa aat act aac aac aca gaa	1588
Thr Glu Asn Ala Val Val Asn Glu Asn Lys Asn Thr Asn Asn Thr Glu	
110 115 120	
aac gat aat agt caa tta aag tta act aat aat gaa caa cca tca gcc	1636
Asn Asp Asn Ser Gln Leu Lys Leu Thr Asn Asn Glu Gln Pro Ser Ala	
125 130 135	
gct act caa gca aac ttg aag aag cta aat cct caa gct gct aag gct	1684
Ala Thr Gln Ala Asn Leu Lys Lys Leu Asn Pro Gln Ala Ala Lys Ala	
140 145 150 155	
ggt caa aat gcc aag att gat gcc ggt agt tta aca gat gat caa att	1732
Val Gln Asn Ala Lys Ile Asp Ala Gly Ser Leu Thr Asp Asp Gln Ile	
160 165 170	
aat gaa tta aat aag att aac ttc tct aag tct gct gaa aag ggt gca	1780
Asn Glu Leu Asn Lys Ile Asn Phe Ser Lys Ser Ala Glu Lys Gly Ala	
175 180 185	
aaa ttg acc ttt aag gac tta gag ggg att ggt aat gct att gtt aag	1828
Lys Leu Thr Phe Lys Asp Leu Glu Gly Ile Gly Asn Ala Ile Val Lys	
190 195 200	
caa gat cca caa tat gct att cct tat tct aat gct aag gaa atc aag	1876
Gln Asp Pro Gln Tyr Ala Ile Pro Tyr Ser Asn Ala Lys Glu Ile Lys	
205 210 215	
aat atg cct gca aca tac act gta gat gcc aca aca ggt aag atg gct	1924
Asn Met Pro Ala Thr Tyr Thr Val Asp Ala Gln Thr Gly Lys Met Ala	
220 225 230 235	

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cat ctt gat gtc tgg gac tct tgg cca gta caa gat cct gtc aca ggt His Leu Asp Val Trp Asp Ser Trp Pro Val Gln Asp Pro Val Thr Gly 240 245 250	1972
tat gta tct aat tac atg ggt tat caa cta gtt att gct atg atg ggt Tyr Val Ser Asn Tyr Met Gly Tyr Gln Leu Val Ile Ala Met Met Gly 255 260 265	2020
att cca aat tgc cca act gga gat aat cat atc tat ctt ctt tac aac Ile Pro Asn Ser Pro Thr Gly Asp Asn His Ile Tyr Leu Leu Tyr Asn 270 275 280	2068
aag tat ggt gat aat gac ttt tct cat tgg cgc aat gca ggt tca atc Lys Tyr Gly Asp Asn Asp Phe Ser His Trp Arg Asn Ala Gly Ser Ile 285 290 295	2116
ttt gga act aaa gaa aca aat gtg ttc caa gaa tgg tca ggt tca gct Phe Gly Thr Lys Glu Thr Asn Val Phe Gln Glu Trp Ser Gly Ser Ala 300 305 310 315	2164
att gta aat gat gat ggt aca att caa cta ttt ttc acc tca aat gat Ile Val Asn Asp Asp Gly Thr Ile Gln Leu Phe Phe Thr Ser Asn Asp 320 325 330	2212
acg tct gat tac aag ttg aat gat caa cgc ctt gct acc gca aca tta Thr Ser Asp Tyr Lys Leu Asn Asp Gln Arg Leu Ala Thr Ala Thr Leu 335 340 345	2260
aac ctt aat gtt gat gat aac ggt gtt tca atc aag agt gtt gat aat Asn Leu Asn Val Asp Asp Asn Gly Val Ser Ile Lys Ser Val Asp Asn 350 355 360	2308
tat caa gtt ttg ttt gaa ggt gat gga ttt cac tac caa act tat gaa Tyr Gln Val Leu Phe Glu Gly Asp Gly Phe His Tyr Gln Thr Tyr Glu 365 370 375	2356
caa ttc gca aac ggc aaa gat cgt gaa aat gat gat tac tgc tta cgt Gln Phe Ala Asn Gly Lys Asp Arg Glu Asn Asp Asp Tyr Cys Leu Arg 380 385 390 395	2404
gac cca cac gtt gtt caa tta gaa aat ggt gat cgt tat ctt gta ttc Asp Pro His Val Val Gln Leu Glu Asn Gly Asp Arg Tyr Leu Val Phe 400 405 410	2452
gaa gct aat act ggg aca gaa gat tac caa agt gac gac caa att tat Glu Ala Asn Thr Gly Thr Glu Asp Tyr Gln Ser Asp Asp Gln Ile Tyr 415 420 425	2500
aat tgg gct aac tat ggt ggc gat gat gcc ttc aat att aag agt tcc Asn Trp Ala Asn Tyr Gly Gly Asp Asp Ala Phe Asn Ile Lys Ser Ser 430 435 440	2548
ttc aag ctt ttg aat aat aag aag gat cgt gaa ttg gct ggt tta gct Phe Lys Leu Leu Asn Asn Lys Lys Asp Arg Glu Leu Ala Gly Leu Ala 445 450 455	2596
aat ggt gca ctt ggt atc tta aag ctc act aac aat caa agt aag cca Asn Gly Ala Leu Gly Ile Leu Lys Leu Thr Asn Asn Gln Ser Lys Pro 460 465 470 475	2644
aag gtt gaa gaa gta tac tca cca ttg gta tct act ttg atg gct tgc Lys Val Glu Glu Val Tyr Ser Pro Leu Val Ser Thr Leu Met Ala Cys 480 485 490	2692
gat gag gta nnn nnn aag ctt ggt gat aag tat tat ctc ttc tcc gta Asp Glu Val Xaa Xaa Lys Leu Gly Asp Lys Tyr Tyr Leu Phe Ser Val 495 500 505	2740
act cgt gta agt cgt ggt tcc gat cgt gaa tta acc gct aag gat aac Thr Arg Val Ser Arg Gly Ser Asp Arg Glu Leu Thr Ala Lys Asp Asn 510 515 520	2788
aca atc gtt ggt gat aac gtt gct atg att ggt tac gtt tcc gat agc Thr Ile Val Gly Asp Asn Val Ala Met Ile Gly Tyr Val Ser Asp Ser 525 530 535	2836
tta atg ggt aag tac aag cca tta aat aac tca ggt gtc gta tta act Leu Met Gly Lys Tyr Lys Pro Leu Asn Asn Ser Gly Val Val Leu Thr 540 545 550 555	2884

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gca tca gta cct gca aac tgg cgt act gct act tat tcc tac tat gca Ala Ser Val Pro Ala Aen Trp Arg Thr Ala Thr Ser Tyr Tyr Ala 560 565 570	2932
gta cct gta gct ggt cat cct gat caa gta tta att act tct tac atg Val Pro Val Ala Gly His Pro Asp Gln Val Leu Ile Thr Ser Tyr Met 575 580 585	2980
agt aac aag gac ttt gct tca ggt gaa gga aac tat gca act tgg gca Ser Asn Lys Asp Phe Ala Ser Gly Glu Gly Asn Tyr Ala Thr Trp Ala 590 595 600	3028
cca agt ttc tta gta caa atc aat cca gat gac acg aca act gta tta Pro Ser Phe Leu Val Gln Ile Aen Pro Asp Asp Thr Thr Thr Val Leu 605 610 615	3076
gca cgt gca act aac caa ggt gac tgg gtg tgg gac gac tct agt cgg Ala Arg Ala Thr Aen Gln Gly Asp Trp Val Trp Asp Asp Ser Ser Arg 620 625 630 635	3124
aac gat aat atg ctc ggt gtt ctt aaa gaa ggt gca gct aac agt gcc Aen Asp Aen Met Leu Gly Val Leu Lys Glu Gly Ala Ala Aen Ser Ala 640 645 650	3172
gcc tta cca ggt gaa tgg ggt aag cca gtt gac tgg agt ttg att aac Ala Leu Pro Gly Glu Trp Gly Lys Pro Val Asp Trp Ser Leu Ile Aen 655 660 665	3220
aga agt cct ggc tta ggc tta aag cct cat caa cca gtt caa cca aag Arg Ser Pro Gly Leu Gly Leu Lys Pro His Gln Pro Val Gln Pro Lys 670 675 680	3268
att gat caa cct gat caa caa cct tct ggt caa aac act aag aat gtc Ile Asp Gln Pro Asp Gln Gln Pro Ser Gly Gln Aen Thr Lys Aen Val 685 690 695	3316
aca cca ggt aat ggt gat aag cct gct ggt aag gca act cct gat aac Thr Pro Gly Asn Gly Asp Lys Pro Ala Gly Lys Ala Thr Pro Asp Aen 700 705 710 715	3364
act aat att gat cca agt gca caa cct tct ggt caa aac act aat att Thr Aen Ile Asp Pro Ser Ala Gln Pro Ser Gly Gln Aen Thr Aen Ile 720 725 730	3412
gat cca agt gca caa mct tct ggt caa aac act aag aat gtc aca cca Asp Pro Ser Ala Gln Xaa Ser Gly Gln Aen Thr Lys Aen Val Thr Pro 735 740 745	3460
ggt aat gag aaa caa ggt aag aat acc gat gca aaa caa tta cca caa Gly Asn Glu Lys Gln Gly Lys Aen Thr Asp Ala Lys Gln Leu Pro Gln 750 755 760	3508
aca ggt aat aag tct ggt tta gca gga ctt tac gct ggt tca tta ctt Thr Gly Aen Lys Ser Gly Leu Ala Gly Leu Tyr Ala Gly Ser Leu Leu 765 770 775	3556
gcc ttg ttt gga ttg gca gca att gaa aag cgt cac gct taa Ala Leu Phe Gly Leu Ala Ala Ile Glu Lys Arg His Ala 780 785 790	3598
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gatagctact cctaaacgtc ctcgaaatcat attcaagcca agaaattcac gcccatgatc	4078
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<210> SEQ ID NO 11
<211> LENGTH: 792
<212> TYPE: PRT
<213> ORGANISM: Lactobacillus reuteri
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (495)..(496)
<223> OTHER INFORMATION: Any amino acid
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (737)
<223> OTHER INFORMATION: Thr or Pro

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<400> SEQUENCE: 11

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  20           25           30
Ser Asn Asn Tyr Asn Gly Val Ala Glu Val Asn Thr Glu Arg Gln Ala
  35           40           45
Asn Gly Gln Ile Gly Val Asp Gly Lys Ile Ile Ser Ala Asn Ser Asn
  50           55           60
Thr Thr Ser Gly Ser Thr Asn Gln Glu Ser Ser Ala Thr Asn Asn Thr
  65           70           75           80
Glu Asn Ala Val Val Asn Glu Ser Lys Asn Thr Asn Asn Thr Glu Asn
  85           90           95
Ala Val Val Asn Glu Asn Lys Asn Thr Asn Asn Thr Glu Asn Ala Val
 100           105           110
Val Asn Glu Asn Lys Asn Thr Asn Asn Thr Glu Asn Asp Asn Ser Gln
 115           120           125
Leu Lys Leu Thr Asn Asn Glu Gln Pro Ser Ala Ala Thr Gln Ala Asn
 130           135           140
Leu Lys Lys Leu Asn Pro Gln Ala Ala Lys Ala Val Gln Asn Ala Lys
 145           150           155           160
Ile Asp Ala Gly Ser Leu Thr Asp Asp Gln Ile Asn Glu Leu Asn Lys
 165           170           175
Ile Asn Phe Ser Lys Ser Ala Glu Lys Gly Ala Lys Leu Thr Phe Lys
 180           185           190
Asp Leu Glu Gly Ile Gly Asn Ala Ile Val Lys Gln Asp Pro Gln Tyr
 195           200           205
Ala Ile Pro Tyr Ser Asn Ala Lys Glu Ile Lys Asn Met Pro Ala Thr
 210           215           220
Tyr Thr Val Asp Ala Gln Thr Gly Lys Met Ala His Leu Asp Val Trp
 225           230           235           240

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Asp Ser Trp Pro Val Gln Asp Pro Val Thr Gly Tyr Val Ser Asn Tyr	245	250	255
Met Gly Tyr Gln Leu Val Ile Ala Met Met Gly Ile Pro Asn Ser Pro	260	265	270
Thr Gly Asp Asn His Ile Tyr Leu Leu Tyr Asn Lys Tyr Gly Asp Asn	275	280	285
Asp Phe Ser His Trp Arg Asn Ala Gly Ser Ile Phe Gly Thr Lys Glu	290	295	300
Thr Asn Val Phe Gln Glu Trp Ser Gly Ser Ala Ile Val Asn Asp Asp	305	310	320
Gly Thr Ile Gln Leu Phe Phe Thr Ser Asn Asp Thr Ser Asp Tyr Lys	325	330	335
Leu Asn Asp Gln Arg Leu Ala Thr Ala Thr Leu Asn Leu Asn Val Asp	340	345	350
Asp Asn Gly Val Ser Ile Lys Ser Val Asp Asn Tyr Gln Val Leu Phe	355	360	365
Glu Gly Asp Gly Phe His Tyr Gln Thr Tyr Glu Gln Phe Ala Asn Gly	370	375	380
Lys Asp Arg Glu Asn Asp Asp Tyr Cys Leu Arg Asp Pro His Val Val	385	390	400
Gln Leu Glu Asn Gly Asp Arg Tyr Leu Val Phe Glu Ala Asn Thr Gly	405	410	415
Thr Glu Asp Tyr Gln Ser Asp Asp Gln Ile Tyr Asn Trp Ala Asn Tyr	420	425	430
Gly Gly Asp Asp Ala Phe Asn Ile Lys Ser Ser Phe Lys Leu Leu Asn	435	440	445
Asn Lys Lys Asp Arg Glu Leu Ala Gly Leu Ala Asn Gly Ala Leu Gly	450	455	460
Ile Leu Lys Leu Thr Asn Asn Gln Ser Lys Pro Lys Val Glu Glu Val	465	470	480
Tyr Ser Pro Leu Val Ser Thr Leu Met Ala Cys Asp Glu Val Xaa Xaa	485	490	495
Lys Leu Gly Asp Lys Tyr Tyr Leu Phe Ser Val Thr Arg Val Ser Arg	500	505	510
Gly Ser Asp Arg Glu Leu Thr Ala Lys Asp Asn Thr Ile Val Gly Asp	515	520	525
Asn Val Ala Met Ile Gly Tyr Val Ser Asp Ser Leu Met Gly Lys Tyr	530	535	540
Lys Pro Leu Asn Asn Ser Gly Val Val Leu Thr Ala Ser Val Pro Ala	545	550	555
Asn Trp Arg Thr Ala Thr Tyr Ser Tyr Tyr Ala Val Pro Val Ala Gly	565	570	575
His Pro Asp Gln Val Leu Ile Thr Ser Tyr Met Ser Asn Lys Asp Phe	580	585	590
Ala Ser Gly Glu Gly Asn Tyr Ala Thr Trp Ala Pro Ser Phe Leu Val	595	600	605
Gln Ile Asn Pro Asp Asp Thr Thr Val Leu Ala Arg Ala Thr Asn	610	615	620
Gln Gly Asp Trp Val Trp Asp Asp Ser Ser Arg Asn Asp Asn Met Leu	625	630	635
Gly Val Leu Lys Glu Gly Ala Ala Asn Ser Ala Ala Leu Pro Gly Glu	645	650	655
Trp Gly Lys Pro Val Asp Trp Ser Leu Ile Asn Arg Ser Pro Gly Leu			

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660	665	670
Gly Leu Lys Pro His Gln Pro Val Gln Pro Lys Ile Asp Gln Pro Asp 675 680 685		
Gln Gln Pro Ser Gly Gln Asn Thr Lys Asn Val Thr Pro Gly Asn Gly 690 695 700		
Asp Lys Pro Ala Gly Lys Ala Thr Pro Asp Asn Thr Asn Ile Asp Pro 705 710 715 720		
Ser Ala Gln Pro Ser Gly Gln Asn Thr Asn Ile Asp Pro Ser Ala Gln 725 730 735		
Xaa Ser Gly Gln Asn Thr Lys Asn Val Thr Pro Gly Asn Glu Lys Gln 740 745 750		
Gly Lys Asn Thr Asp Ala Lys Gln Leu Pro Gln Thr Gly Asn Lys Ser 755 760 765		
Gly Leu Ala Gly Leu Tyr Ala Gly Ser Leu Leu Ala Leu Phe Gly Leu 770 775 780		
Ala Ala Ile Glu Lys Arg His Ala 785 790		
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ctgataataa tggaaatgta tcac		24
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<220> FEATURE:		
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catgatcata agtttggtag taatag		26
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gtgatcatt tccattatta tcag		24
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<212> TYPE: DNA		
<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer		
 <400> SEQUENCE: 15		
ctattactac caaacttatg atcatg		26
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<212> TYPE: DNA		
<213> ORGANISM: Artificial Sequence		

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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 16

ccatggccat ggtagaacgc aaggaacata aaaaaatg                               38

<210> SEQ ID NO 17
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 17

agatctagat ctgttaaatc gacgtttgtt aatttctg                               38

<210> SEQ ID NO 18
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<220> FEATURE:
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<223> OTHER INFORMATION: a, c, t, g, other or unknown

<400> SEQUENCE: 18

gaygtntggg aywntgggc c                                                  21

<210> SEQ ID NO 19
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<220> FEATURE:
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<400> SEQUENCE: 19

gtngcnawnc cnawccayts ytg                                              23

<210> SEQ ID NO 20
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 20

gaatgtaggt ccaatttttg gc                                              22

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<210> SEQ ID NO 21
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 <220> FEATURE:
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<400> SEQUENCE: 21

cctgtccgaa catcttgaac tg

22

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 <223> OTHER INFORMATION: a, c, t, g, other or unknown

<400> SEQUENCE: 22

arraanswng gngcvmangt naw

23

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<400> SEQUENCE: 23

tayaayggng tngcngargt naa

23

<210> SEQ ID NO 24
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 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: Primer

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<400> SEQUENCE: 24

ccgaccatct tgtttgatta ac

22

<210> SEQ ID NO 25

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 25

aaytataayg gygttgcrvg aagt

24

<210> SEQ ID NO 26

<211> LENGTH: 21

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (9)

<223> OTHER INFORMATION: a, c, t, g, other or unknown

<400> SEQUENCE: 26

taccgnwanc tacttcaact t

21

<210> SEQ ID NO 27

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Lactobacillus reuteri

<400> SEQUENCE: 27

Tyr Asn Gly Val Ala Glu Val Lys Lys Arg Gly Tyr Phe Tyr Ala Arg
1 5 10 15

Thr

<210> SEQ ID NO 28

<211> LENGTH: 17

<212> TYPE: PRT

<213> ORGANISM: Lactobacillus reuteri

<400> SEQUENCE: 28

Tyr Asn Gly Val Ala Glu Val Asn Thr Glu Arg Gln Ala Asn Gly Gly
1 5 10 15

Ile

<210> SEQ ID NO 29

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Bacillus amyloliquefaciens

<400> SEQUENCE: 29

Gly Leu Asp Val Trp Asp Ser Trp Pro Leu Gln Asn Ala Asp
1 5 10

<210> SEQ ID NO 30

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 30

Gly Leu Asp Val Trp Asp Ser Trp Pro Leu Gln Asn Ala Asp

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1 5 10

<210> SEQ ID NO 31
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: Streptococcus mutans
 <400> SEQUENCE: 31
 Asp Leu Asp Val Trp Asp Ser Trp Pro Val Gln Asp Ala Lys
 1 5 10

<210> SEQ ID NO 32
 <211> LENGTH: 14
 <212> TYPE: PRT
 <213> ORGANISM: Streptococcus salivarius
 <400> SEQUENCE: 32
 Glu Ile Asp Val Trp Asp Ser Trp Pro Val Gln Asp Ala Lys
 1 5 10

<210> SEQ ID NO 33
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Bacillus amyloliquefaciens
 <400> SEQUENCE: 33
 Gln Thr Gln Glu Trp Ser Gly Ser Ala Thr Phe Thr Ser Asp Gly Lys
 1 5 10 15

<210> SEQ ID NO 34
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 <212> TYPE: PRT
 <213> ORGANISM: Bacillus subtilis
 <400> SEQUENCE: 34
 Gln Thr Gln Glu Trp Ser Gly Ser Ala Thr Phe Thr Ser Asp Gly Lys
 1 5 10 15

<210> SEQ ID NO 35
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Streptococcus mutans
 <400> SEQUENCE: 35
 Leu Thr Gln Glu Trp Ser Gly Ser Ala Thr Val Asn Glu Asp Gly Ser
 1 5 10 15

<210> SEQ ID NO 36
 <211> LENGTH: 16
 <212> TYPE: PRT
 <213> ORGANISM: Streptococcus salivarius
 <400> SEQUENCE: 36
 Asp Asp Gln Gln Trp Ser Gly Ser Ala Thr Val Asn Ser Asp Gly Ser
 1 5 10 15

<210> SEQ ID NO 37
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Bacillus amyloliquefaciens
 <400> SEQUENCE: 37
 Lys Ala Thr Phe Gly Pro Ser Phe Leu Met Asn
 1 5 10

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<210> SEQ ID NO 38
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: *Bacillus subtilis*
 <400> SEQUENCE: 38
 Gln Ser Thr Phe Ala Pro Ser Phe Leu Leu Asn
 1 5 10

 <210> SEQ ID NO 39
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: *Streptococcus mutans*
 <400> SEQUENCE: 39
 Asn Ser Thr Trp Ala Pro Ser Phe Leu Ile Gln
 1 5 10

 <210> SEQ ID NO 40
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: *Streptococcus salivarius*
 <400> SEQUENCE: 40
 Lys Ser Thr Trp Ala Pro Ser Phe Leu Ile Lys
 1 5 10

What is claimed is:

1. A process of producing a fructo-oligosaccharide or fructo-polysaccharide, having $\beta(2-1)$ linked D-fructosyl units comprising forming a mixture by combining sucrose with at least one reaction partner selected from the group consisting of:

a) a protein having fructosyltransferase activity, which exhibits at least 85% amino acid identity, as determined by a BLAST algorithm, with an amino acid sequence of SEQ ID No. 1, and

b) a recombinant host cell containing one or more copies of a nucleic acid construct encoding for said protein (a) and capable of expressing said protein;

wherein said reaction partner interacts with sucrose to produce a fructo-oligosaccharide or fructo-polysaccharide.

2. The process according to claim 1, wherein said protein is a recombinant protein.

3. A process according to claim 1, further comprising chemically modifying said fructo-oligosaccharide or fructo-polysaccharide by simultaneous 3- and 4-oxidation, 1- or 6-oxidation, phosphorylation, acylation, hydroxyalkylation, carboxymethylation or amino-alkylation of one or more anhydrofructose units, or by hydrolysis.

4. The process according to claim 1, further comprising adding a food or beverage composition to said mixture to obtain a prebiotic composition.

5. The process according to claim 1, further comprising adding to said mixture a *Lactobacillus* strain capable of producing an oligosaccharide or polysaccharide and optionally a food or beverage composition, to obtain a synbiotic composition.

6. A process of producing a fructo-oligosaccharide or fructo-polysaccharide, having $\beta(2-1)$ linked D-fructosyl units comprising combining sucrose and a protein to form a mixture, said protein having fructosyltransferase activity, which exhibits at least 85% amino acid identity, as determined by a BLAST algorithm, with an amino acid sequence of SEQ ID No. 1, and

interacting said sucrose with said protein to produce said fructo-oligosaccharide or fructo-polysaccharide.

7. A process for producing a fructo-oligosaccharide or fructo-polysaccharide, having $\beta(2-6)$ linked D-fructosyl units comprising forming a mixture by combining sucrose with a reaction partner, wherein said reaction partner is a recombinant host cell containing one or more copies of a nucleic acid construct encoding for a protein having fructosyltransferase activity, which exhibits at least 85% amino acid identity, as determined by a BLAST algorithm, with an amino acid sequence of SEQ ID No. 11, and wherein said reaction partner interacts with sucrose to provide a fructo-oligosaccharide or fructo-polysaccharide.

8. A process according to claim 7, further comprising chemically modifying said fructo-oligosaccharide or fructo-polysaccharide by simultaneous 3- and 4-oxidation, 1- or 6-oxidation, phosphorylation, acylation, hydroxyalkylation, carboxymethylation or amino-alkylation of one or more anhydrofructose units, or by hydrolysis.

* * * * *